

DETERMINATION OF ACUTE TOXICITY OF UREA TO *EISENIA FETIDA* BY A SIMPLE PAPER CONTACT METHOD

***K. S. Abhiramy and P. Ronald Ross**

*Research Scholar, Department of Zoology, Faculty of Science, Annamalai University,
Annamalainagar – 608002, Tamilnadu, India

Assistant Professor, Zoology Wing, Faculty of Science, Annamalai University,
Annamalainagar – 608 002, Tamilnadu, India

Emails: *umak.senthil@gmail.com, r_ross1971@yahoo.co.in (*Corresponding Author)

Abstract: Urea is the most commonly used nitrogen fertilizer worldwide. The overused urea in agricultural fields may affect the soil organisms especially, the earthworms which are known well for their role in soil fertility. Earthworms are typically inferred as soil indicators without which soil fertility cannot be measured directly. An acute toxicity test of urea to earthworm (*Eisenia fetida*) was performed using a simple paper contact method proposed by OECD (Organization for Economical Cooperation and Development) testing guideline no. 207. This is a simple screening test to identify the toxic potential of the chemical to earthworm. The lethal concentration was recorded as $28\mu\text{g}/\text{cm}^2$. Based on the resulting LC_{50} value, urea was categorized as “very toxic” to earthworm according to Roberts and Dorrough (1984). The result of this study demonstrates that inorganic fertilizers can also be toxic to earthworms when contacted directly. Thus there arises an unavoidable need for monitoring the usage of fertilizer dosage on agricultural lands, particularly the urea.

Keywords: Urea; Acute toxicity; 48 h LC_{50} ; *Eisenia fetida*; Paper contact method.

Introduction

The post era of green revolution has led to environmental pollution due to excessive use of agrochemicals and fertilizers and thus threatened the fragile ecosystem. Indian farmers have adopted the green revolution technology for the past 3 decades leading to the exploitation of agricultural lands. Fertilizers are intentionally utilized in agriculture in huge tonnages each year in order to enrich soils artificially to sustain food production. The general recommendation for urea is 120kg/hectare in agricultural lands as per the Indian soil testing manual released in 2011 by the Department of Agriculture, Ministry of Agriculture, India. But Indian farmers overuse urea to achieve more productivity ignoring the negative effects on soil organisms particularly the earthworms. This situation actually prevails all over the world.

Recently much attention is paid to soil health and environmental safety. Earthworms play an important role in soil fertility and they constitute the 60% of the soil macrofauna biomass

(Barlett et al., 2010). They are extremely important in soil formation, principally by consuming organic matter, fragmenting it, and mixing it intimately with soil mineral particles to form water-stable aggregates. Thus it is important for the presence of earthworms in the soil to maintain its structure and function, particularly in agricultural fields.

Because of their natural habitat, earthworms are exposed to a variety of chemicals like chemical fertilizers, pesticides, insecticides, etc... in agricultural fields and therefore are a suitable indicators for the assessment of toxicity of chemicals. Although effects of urea on plant growth and soil biota communities are studied in detail, there is no data about its effects on earthworms. Ma et al., (1990) studied long-term effects (20 y) on nitrogenous fertilizers usage on lumbricid earthworms in soil. Only single and joint effects of acetochlor and urea on earthworm *E.fetida* populations in phaeozem was studied by Xiao et al., (2004). Urea is also an artificially manufactured chemical and could affect earthworms due to their common usage in the agriculture. Since the data about its effects on earthworms are scarce, the aim of this study was to determine the toxic effects of urea on the earthworm *E.fetida*.

Ecological toxicity of urea on earthworm, *E.fetida* was thus studied using a simple paper contact method. Paper contact method was proposed by OECD (1984) as a screening test to prove the toxic potential of chemical. This method has been successively adopted by many scientists (Karanjkar and Naik, 2010; Miyazaki et al., 2002; Roberts and Dorough, 1984; Wang et al., 2012; Velki and Hackenberger, 2013) to prove the toxicity of chemical to earthworms. But the chemicals hereby studied are pesticides and herbicides and no work has been done in chemical fertilizers. This is the first toxicity study made on chemical fertilizer using this methodology. Roberts and Dorough (1984) tested the toxicity of 90 chemicals on *E.fetida* using the simple paper contact method and classified the chemicals as supertoxic, extremely toxic, very toxic, moderately toxic and relatively non-toxic. This was the pioneer study made adopting this method and here the same criterion was followed.

Materials and Method

Earthworms

E.fetida was adopted as the test species, because it is the recommended species in OECD (1984) guideline for testing of chemicals no. 207, earthworm, acute toxicity tests. The earthworms were purchased from the vermicomposting unit, Annamalai University, Annamalainagar. They were all cultured under the same conditions, fed mainly on the excrement of milk cows. This culture was judged to be free for contaminants. Adult

earthworms, which possessed clitellae and had an individual wet weight of 200 ± 30 mg (after voiding the gut content), were selected for testing.

Test chemicals and solutions

The commonly used chemical fertilizer, urea was used as test chemical. It was purchased from the Agriculture office, Annamalainagar, Cuddalore district, Tamilnadu, India. Aqueous solutions of various concentrations were prepared by dissolving the urea in deionized water. The concentrations were prepared in mg/ml and the toxicity was measured as $\mu\text{g}/\text{cm}^2$.

Acute toxicity test

An acute toxicity test of urea was performed on earthworms using a simple paper contact method proposed by OECD testing guideline no. 207. This is a simple screening test to identify the toxic potential of the chemical to earthworm. The test vial was a plastic petri dish (Wang et al., 2012) of 14cm diameter and 2cm height. Round filter paper (Whatman No. 1) of size 8.5cm diameter was cut to the suitable size and placed in such a way that sides are lined with filter paper. Five concentrations were prepared by dissolving urea in 5ml deionized water in a geometric series (5, 10, 20, 40 and 80mg) and were pipetted into each vial in order to wet the filter paper. Blank tests were performed with 5ml of deionized water only. For each treatment, ten replicates were used, each consisting of one earthworm per vial. Adult earthworms, which possessed clitellum and had an individual wet weight of 250–350mg, were selected for testing. Earthworms were washed briefly with deionized water, and were kept on moist filter paper for 3h to devoid the gut content, after which it was rinsed again with deionized water, blotted on the filter paper and placed in a test vial (One earthworm per vial). After the introduction of earthworm the vial was covered with plastic film that had been punched with small holes using needles. Tests were done in the dark at $28 \pm 2^{\circ}\text{C}$ for 48 h. After 48 hours the earthworm was monitored for mortality by a gentle mechanical stimulus to the front part.

Statistical analysis

For the filter paper contact method, the toxicity is expressed in $\mu\text{g}/\text{cm}^2$. Based on the resulting 48-h LC_{50} values, the urea fertilizer will be classified as supertoxic ($<1.0 \mu\text{g}/\text{cm}^2$), extremely toxic ($1-10 \mu\text{g}/\text{cm}^2$), very toxic ($10-100 \mu\text{g}/\text{cm}^2$), moderately toxic ($100-1000 \mu\text{g}/\text{cm}^2$) or relatively nontoxic ($>1000 \mu\text{g}/\text{cm}^2$) (Roberts and Dorough, 1984).

Results and Discussion

The lethal toxic concentration of urea to *E.fetida* was evaluated as $28\mu\text{g}/\text{cm}^2$. Thus the relative toxicity grade of urea was categorized as “very toxic” to *E.fetida*. With uniform area of contact exposure of urea to the earthworm in different concentrations showed various toxic effects in filter paper substrate medium. A geometric concentration series of test solution (5, 10, 20, 40 and 80mg/5ml) was prepared and tested, in which mortality of earthworms was observed in 20, 40 and 80mg concentrations after 28, 16 and 8h respectively. Only the 5mg/5ml concentration was non-toxic to earthworm for 48 h. The lethal concentration for 48h to *E.fetida* was 10mg/5ml concentration (Figure 1). The deleterious effects of urea on earthworm were lesions, inflammations and separation of the posterior body parts (Figure 2). The worm was not able to move after 40h, neural retention starts and the body parts separate leading to death.



Figure 1: Mortality of *E.fetida* at 10mg/5ml concentration of urea



Figure 2: Detrimental effects like lesions, inflammations and separation of body parts observed on *E.fetida*

Contact filter paper test is an initial screening technique to assess the relative toxicity of chemicals to earthworms. In this initial screening test, the chemicals are absorbed into the earthworm body mainly through the skin when it is moving around the filter paper. Though it fails to represent the situation in soil, it is important to know the toxic status of a particular chemical, whether it is toxic or not. If the chemical proved to be toxic, further extended study on artificial soil can be carried out. If the chemical proved to be non-toxic, there is no need for the extended study. It has been demonstrated for many decades that most of the inorganic mineral fertilizers are non-toxic to earthworms; however, it has been disproved in this study.

Conclusion

From the above results, it may be concluded that the acute toxicity of urea on *E.fetida* by using a simple paper contact method was really significant in confirming the toxic potential. The application of environmentally realistic doses of urea revealed the possible harmful effects on earthworms when contact directly. Thus, in future this method will be necessary to find a way to determine the sensitivity of the earthworm's acute toxicity before going for the evaluation in soil (i.e. acute and chronic toxicity tests in artificial soil). Soil being a very complex system, it is often difficult to compare toxicity data directly. Though the paper contact method ignores the contribution of soil components it has high advantages of reproducibility and the possible of direct comparison of results. Using the data obtained from this method, the range of test concentration for acute and chronic toxicity tests can also be determined.

References

- [1] Bartlett, M.D., Briones, M.J.I., Neilson, R., Schmidt, O., Spurgeon, D., and Creamer, R.E. (2010): A critical review of current methods in earthworm ecology: from individuals to populations, *Eur. J. Soil Biol*, 46: 67–73.
- [2] Karanjkar, A.S., and Naik, R.L. (2010): Acute Toxicity: Novel Mode of Pesticides on Earthworm, *International Journal of Plant Protection*, 2(2): 182-185.
- [3] Ma, W.C., Brussaard, L., and De Ridder, J.A. (1990): Long-term Effects of Nitrogenous Fertilizers on Grassland Earthworms (*Oligochaeta: Lumbricidae*): Their Relation to Soil Acidification, *Agric.Ecosystem Env*, 30: 71-80.
- [4] Miyazaki, A., Amano, T., Saito, H., and Nakano, Y. (2002): Acute toxicity of chlorophenols to earthworms using a simple paper contact method and comparison with toxicities to fresh water organisms, *Chemosphere*, 47: 65-69.
- [5] OECD, (1984): Organization for Economical Cooperation and Development Guideline for Testing of Chemicals, No. 207, Earthworm Acute Toxicity, OECD, Paris, France.
- [6] Roberts, B.L., and Dorough, H.W. (1984): Relative toxicities of chemicals to the earthworm *E.fetida*, *Environ. Toxicol. Chem*, 3: 67–78.
- [7] Velki, M., and Hackenberger, B.K. (2013): Biomarker responses in earthworm *E.fetida* to pirimiphos-methyl and deltamethrin using different toxicity tests, *Chemosphere*, 90: 1216-1226.

- [8] Wang, Y., Cang, T., Zhao, X., Yu, R., Chen, L., Wu, C., and Wang, Q. (2012): Comparative acute toxicity of twenty-four insecticides to earthworm, *E.fetida*, *Ecotoxicol Environ Saf*, 79: 122-128.
- [9] Xiao, H., Zhou, Q.X., and Liang, J.D. (2004), Single and joint effects of acetochlor and urea on earthworm *E.fetida* populations in phaozem, *Environmental Geochemistry and Health*, 26: 277–283.